Melittin-induced fusion of acidic liposomes

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Melittin-induced fusion of acidic liposomes. Fusion was observed in the electron-microscope and assayed as intermixing of both liposomes' contents and membranes. The melittin concentrations required for fusion induction were in the μM range compared to over 10 mM Ca^{2+} required for a comparable effect. It is suggested that the high efficiency of melittin is due to its amphipathic nature. Its hydrophobic moiety is probably anchored in one liposome while the positively charged hydrophilic moiety attracts another liposome.

Fusion *Liposome* *Melittin* *Cardiolipin*

1. INTRODUCTION

Liposome–liposome and liposome–cell fusion have been subjects of intensive research for the past decade (see reviews [1,2]). Membrane fusion is an ubiquitous and essential mechanism in cell biology. Valuable information leading to better understanding of fusion phenomena occurring in cells has been gained from studies of artificial lipid model systems; e.g., liposomes. However, liposome–cell interactions have been studied mainly for practical aims. Liposomes have been envisaged as delivery vehicles for drugs and macromolecules into cells both in vivo and in vitro (see reviews [3,4]). Fusion of liposomes and reconstituted proteoliposomes with planar lipid membranes is a procedure gaining wider popularity for insertion of proteins and antigens into planar membranes [5,6].

A widely used fusion system is the cation-induced fusion of acidic liposomes [1,2]. [Ca^{2+}] in the mM range induce fusion of liposomes containing a variety of acidic lipids. Polycations (e.g., polylysine) induce fusion at relatively lower concentrations [7]. Here, we demonstrate that melittin is an extremely efficient fusogen. It induced fusion of liposomes at concentrations two orders of magnitude lower than those needed when calcium or magnesium was used.

Melittin constitutes half of the protein content of bee venom. It is a well-known amphipathic peptide which acts as direct lytic factor on artificial and biological membranes. It has been the subject of much research dedicated to protein–membrane interaction [8–10]. Here, we demonstrate that melittin induced fusion with little effect on the liposomes' integrity and only at higher concentrations did it lyse the vesicles. Melting neutral liposomes composed of saturated phospholipids in presence of melittin induced their fusion [11].

2. MATERIALS AND METHODS

Cardiolipin, egg yolk phosphatidylcholine (type VII) and melittin were purchased from Sigma, EDTA (10 mM) was included in some of the experiments in order to exclude the possibility that phospholipase A contaminating the melittin sample had effect on liposome fusion. Polymyxin B and poly(L-lysine) (M_r 70000) were purchased both from Chemalog and from Sigma. Phosphatidylethanolamine was purified from soybean phospholipids as in [12].

Small unilamellar liposomes were prepared by sonication as in [13]. The buffer used throughout the work consisted of 30 mM *N*-2 hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes, pH 7.6), 128 mM KCl and 32 mM NaCl. The assay of

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membranes intermixing was based on dilution of chlorophylls and was performed as described. Intermixing of vesicle contents was determined by the procedure in [15] as modified in [16]. Leakage of vesicle contents was determined with the fluorescent dye carboxyfluorescein as in [17]. Samples were prepared for electron-microscopy as in [7].

3. RESULTS

Melittin induced fusion of acidic liposomes. The fusion was assayed as intermixing of both liposomes' contents and membranes. In order to assay membrane intermixing, two liposome populations were incubated together, one containing chlorophyll and the other non-pigmented. Upon addition of melittin, fusion of liposomes occurred with dilution of chlorophyll in the membranes. The dilution was monitored as reduction in energy transfer from chlorophyll *b* to chlorophyll *a* [14]. As shown in fig.1a, already at melittin concentrations of 1 μM, appreciable fusion was observed. Maximal fusion occurred at concentrations of 50–100 μM.

Intermixing of vesicle contents was monitored using the assay developed in [15]. Two liposome populations were mixed, one containing terbium ions and the other dipicolinic acid. Upon addition of melittin, liposome fusion occurred resulting in formation of the fluorescent complex terbium–dipicolinic acid. Fusion could be assayed at melittin concentrations of about 1 μM. Maximal fusion was obtained at a concentration range of about 50 μM. Higher concentrations caused rapid leakage of vesicle contents into the medium where the fluorescence was quenched by EDTA.

Leakage of vesicle contents was monitored as dequenching of trapped carboxyfluorescein [17]. High concentrations of the dye were trapped within the liposomes. Under these conditions, the fluorescence of the dye was almost completely quenched. Upon leakage of the dye, it was diluted and became highly fluorescent. As shown in fig.1, at low concentrations melittin induced fusion with very little leakage of the contents, while at high concentrations membrane fusion was maximal but vesicle contents leaked out. At concentrations of about 50 μM, efficient fusion of vesicles occurred resulting in efficient intermixing of vesicle contents with little leakage of the contents.

As shown in fig.2, melittin was an extremely ef-

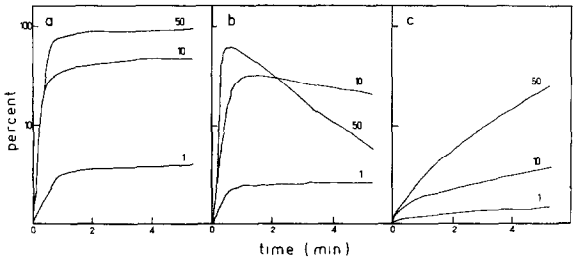

Fig.1. Melittin-induced fusion of liposomes. Small unilamellar liposomes containing phosphatidylethanolamine, phosphatidylcholine and cardiolipin (50, 20 and 30%, respectively) were prepared and incubated at final concentrations of 1, 10 or 50 μM. Fusion and leakage of vesicle contents were assayed as described in section 2. Part a: fusion of membranes was assayed as dilution of chlorophyll included in part of the liposome population. Part b: intermixing of liposome contents was assayed as fluorescence of Tb–dipicolinic acid complex. Part c: leakage of liposome contents was assayed as dilution of trapped carboxyfluorescein. All the results were expressed as the % of maximal fusion or leakage.

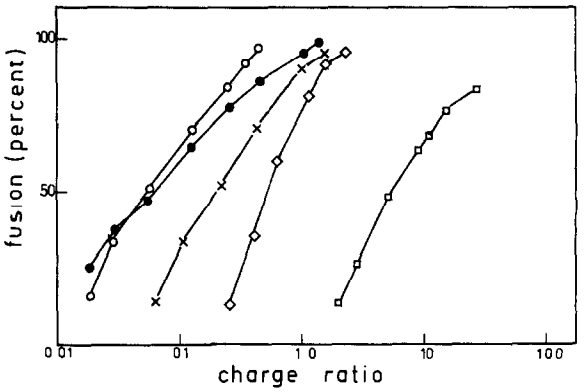

Fig.2. Efficiency of various cations in inducing fusion. Liposomes containing a chlorophyll extract (1%) and a similar phospholipid composition described in the legend to fig.1 were prepared and used at a final concentration of 0.5 mg/ml. Various concentrations of Ca^{2+} (□—□), La^{3+} (◇—◇), poly-L-lysine (M_r 70000, ×—×), polymyxin B (○—○) and melittin (●—●) were added and fusion was assayed after 5 min incubation at room temperature. Charge ratios were calculated assuming that all charged groups of the cations and cardiolipin were fully charged. The distribution of cardiolipin across the liposome membranes was ignored.

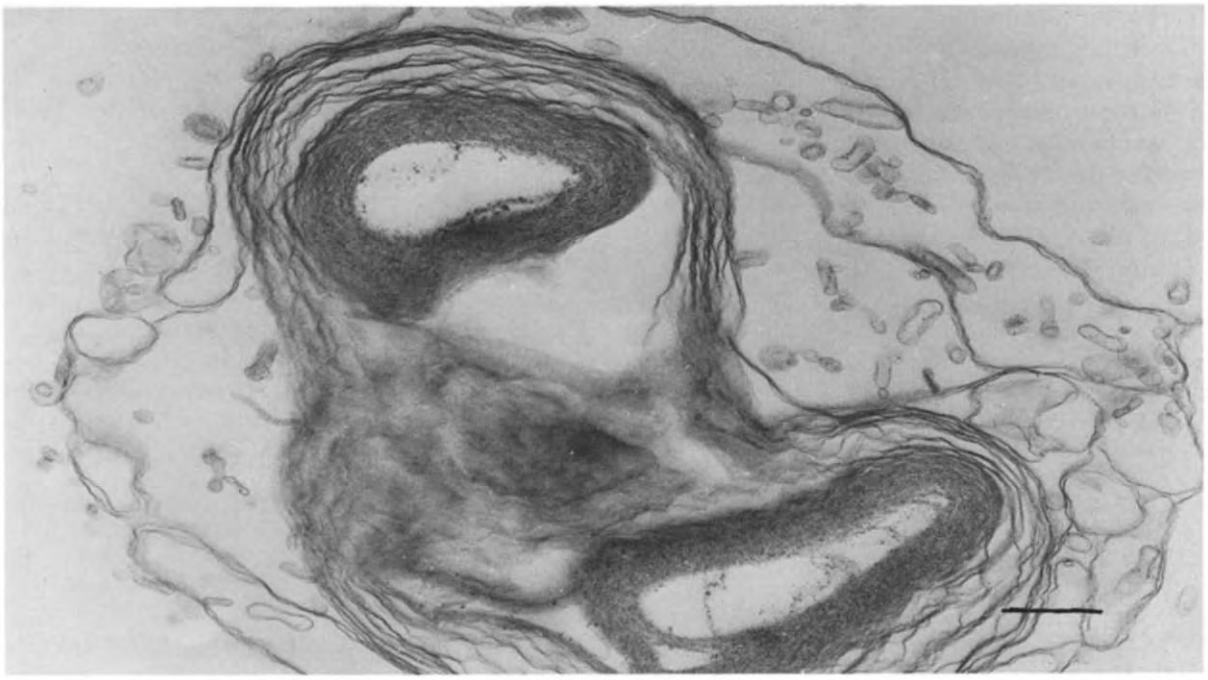

Fig.3. Electron-micrograph of fused liposomes. Small unilamellar liposomes were fused as described in the legend to fig.1. The melittin concentration was 50 μM. The bar's length is equivalent to 0.5 μm.

ficient inducer of fusion. For comparison purposes, concentrations of the various cations was expressed as ratios of their charge to that of the liposomes. Ca^{2+}, the most widely used fusogens, were effective at charge ratios of 3–30. La^{3+} induced fusion at a charge ratio of about 0.5, while polylysine was effective at 0.08–0.5. Melittin induced fusion already at charge ratios of 0.02. As reported in [14], a similar efficiency was exhibited by polymyxin B which induced fusion at charge ratios of 0.02–0.2.

Electron-microscopy confirmed that melittin induced fusion of vesicles. Liposomes were prepared by sonication. Their diameter was in the range of 30–60 nm. After melittin-induced fusion large structures composed of bilayers were formed. As shown in fig.3, even at high melittin:lipid ratios, the bilayer structure was not destroyed and the fused vesicles had assumed the organization of a large multilamellar vesicle.

4. DISCUSSION

Melittin induced fusion at very low concentrations. 100–1000-fold higher concentrations of calcium or magnesium ions were required in order to induce a comparable effect. Polylysine concentrations required were 10-times higher, while polymyxin B induced fusion at comparable concentrations. Both polymyxin B and melittin are amphipathic molecules with a hydrophobic moiety and a hydrophylic one bearing 5 positive charges [8–10,18]. It sees that the combination of a hydrophobic tail anchoring the molecule in the membrane and a hydrophilic moiety capable of electrostatic interaction are the characteristics rendering these molecules so efficient in membrane fusion. It is possible that the molecules adhere and integrate into one liposome and subsequently attract another with their exposed positive charges. Poly(L-lysine), La^{3+}, polymyxin B and melittin at concentrations higher than those required for fusion, lyse cells and disrupt membranes [8–10,19,20]. At the concentrations required for fusion, the disruptive effect of these agents is low. It has been suggested in the past that a necessary intermediate step in membrane fusion is destabilization of the membranes. It is possible

that the destabilizing effect of these agents is one of the factors rendering them efficient in promoting fusion.

The melittin concentrations required for promotion of liposome fusion are low and probably comparable or even lower than those injected by bees. The possibility that the inflammatory effect of bee venom is mediated by promotion of membrane fusion should be considered.

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